Name or type of instrument	**Tims-Tof-fleX MALDI-2 (High-speed and high spatial resolution MALDI mass spectrometry imaging instrument with ion mobility)**
Unit	Spatial Mass Spectrometry
Platform	Spatial and Single Cell Biology
Applicant (Head of Unit)	Anna Nilsson
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Instrument, acquisition cost (kSEK)	13 260
Funding applied for (kSEK)	3 660

Description of the instrument

We aim to purchase the newly released tims-TOF fleX MALDI-2 instrument equipped with a novel two-laser technology MALDI source enabling increased sensitivity and improved spatial resolution (https://www.bruker.com/en/products-and-solutions/mass-spectrometry/timstof/timstof-flex-maldi-2.html). Three units from the Spatial and Single Cell Biology platform recently received a VR-Infrastructure grant (Spatial Omics). The Spatial Mass Spectrometry unit was funded with 9.6 MSEK, which will cover most of the cost for the instrument, but additional funding is required to cover the full cost due to price increases and instrument technology upgrades (see 'Statement of need').

Prof. Per Andrén's laboratory at UU has two state-of-the-art 7 Tesla MALDI-Fourier transform ion cyclotron resonance (FTICR) instruments (installed in 2018 and 2020, 10 MSEK/each), one MALDI time-of-flight (TOF)-TOF instrument (installed in 2014, 4 MSEK), one triple quadrupole mass spectrometer equipped with a desorption electrospray ionization (DESI) imaging source (installed in 2021). These instruments enable imaging with high mass specificity and high sensitivity.

The new tims-TOF fleX MALDI-2 instrument increases the sample through-put by imaging at high-speed (15 pixels/s) and it substantially improves the spatial (lateral) resolution to 5 µm on a tissue section, whereas our current MALDI-FTICR instruments typically acquire spectra at 1 pixels/s at 20 µm lateral resolution. Furthermore, it improves on-tissue identification and characterization of molecules by its trapped ion mobility separation (TIMS) feature. TIMS provides fast orthogonal separation that efficiently unravels complex mass spectra where each mass-to-charge (m/z) ion can contain many overlapping features. The result is not only the ability to extract information from single isobars, but also exact masses that have different ion mobilities. Furthermore, the MALDI-2 source consists of a novel two-laser technology that increases the sensitivity by up to three orders of magnitude depending on the analyte and allows near single cell resolution.

The instrument has recently (summer 2022) been upgraded with a new type of module for the MALDI source called microGRID. The microGRID improves the MALDI stage to sub-micron precision to correct laser positioning on tissue surfaces down to 5 µm, virtually eliminating any visual artefacts or artefacts in co-registration of MALDI images with optical microscopy (or other imaging modality) (Fig 1). With the new instrument, which is a considerable faster imaging instrument than the ones we

currently use, we expect to increase the number of projects, project throughput and quality of the results. There is no other national service (or in Scandinavia) offering this technology.

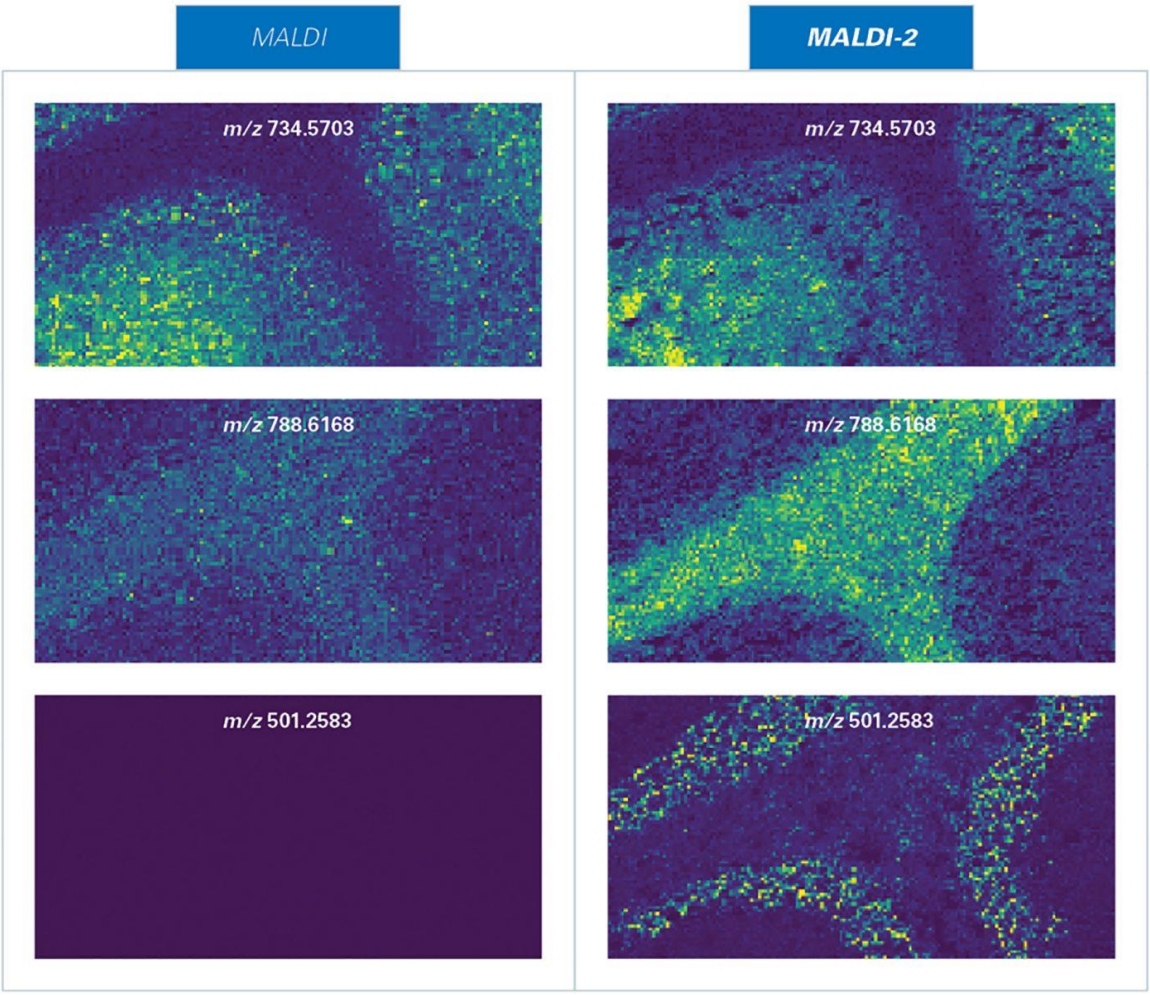

Figure 1. Complementing microGRID imaging with MALDI-2 post-ionization enhances the sensitivity detecting even the finest molecular distributions (from https://www.bruker.com/en/products-and-solutions/mass-spectrometry/timstof/timstof-flex-maldi-2.html).

Statement of need

Three units from the Spatial and Single Cell Biology platform recently received a VR-Infrastructure grant (no. 2021-00189, total 29 434 kSEK). The Spatial Mass Spectrometry unit received **9 600 kSEK** for a new mass spectrometry imaging (MSI) instrument (i.e., tims-TOF fleX MALDI-2). This amount was based on a quote from Bruker Daltonics in the end of 2021. Since then, the price has increased to 13 260 kSEK due to the microGRID technology upgrade, as well as additional price increases. ***Therefore, this extra funding is crucial to cover the increased cost.***

The tims-TOF fleX MALDI-2 instrument will increase the throughput of our projects due to the significantly reduced analysis time *per* tissue section. It will increase our possibility to detect and identify more molecules in tissue sections because of the instrument's increased sensitivity and its ability to separate isobaric species.

The aim is also to develop and provide a new technology based on novel photocleavable mass-tags (PC-MTs) that enable highly multiplexed (HiPLEX) immunohistochemistry (IHC) analysis of proteins in tissue sections, i.e., simultaneous mapping of hundreds of targeted proteins based on tims-TOF fleX MALDI-2 analysis. We will explore the capabilities of this innovative approach to provide more powerful methods for researchers to study cellular-level spatial distributions of biomolecules in tissues. This will have potent applications in diverse fields, including proteomics, tissue pathology, tissue diagnostics, therapeutics, precision medicine, drug discovery and their therapeutic development.

In addition, we will increase the MALDI-MSI spatial resolution (from 5 µm) to <1 µm by using morphology-guided deep learning, enabling imaging of untargeted and targeted molecules in single cells directly in tissue sections. IHC and MALDI-MSI have not been successfully applied in tandem in analyses of the same tissue sections for mapping small molecules and intact proteins to date, but the combination offers exciting opportunities for multiomic characterization of healthy and disease-associated tissue states with unprecedented resolution and detail.

These approaches further expand our capability of imaging a wider range of molecules, previously not possible. The MALDI HiPLEX-IHC and morphology-guided deep learning projects have been funded by a Technology Development Project grant from SciLifeLab.